

***Beltrania shenzhenica* sp. nov. from Guangdong, China**

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ABSTRACT—During a survey of saprophytic microfungi on dead leaves from Futian Mangrove Nature Reserve in Guangdong Province, China, a new species of asexual ascomycota, *Beltrania shenzhenica*, was identified based on morphology and phylogenetic analyses of partial gene sequences of ITS and LSU. The detailed morphological description, phylogenetic tree, and photographs are provided.

KEY WORDS—*Amphisphaeriales*, anamorphic fungi, *Beltraniaceae*, phylogeny, taxonomy,

Introduction

Beltrania Penz., typified by *Beltrania rhombica* Penz. (Penzig 1882), is currently classified in *Beltraniaceae*, *Amphisphaeriales* (Wijayawardene & al. 2020, Zheng & al. 2020). The genus was mainly characterized by mostly unbranched dark setae with radially lobed basal cells, macronematous conidiophores arising from basal cells of setae or from separate radially lobed basal cells, and polyblastic, integrated, terminal, sympodial and denticulate conidiogenous cells that produce solitary acropleurogenous swollen separating cells and biconic, spicate or apiculate conidia with a hyaline equatorial band (Ellis 1971, Seifert & al. 2011, Lin & al. 2017, Hyde & al. 2020). *Beltrania* contains twenty species, of which eight are represented by molecular data (Harkness 1884, Wakefield 1931, Hughes 1951, Pirozynski & Patil 1970, Matsushima 1975, Rao & Varghese 1978, Zhang & Zhang 2003,

Crous & al. 2014, Tibpromma & al. 2018, Bandgar & Patil 2019, Hyde & al. 2020, Zheng & al 2020).

Futian Mangrove Nature Reserve, located northeast of Shenzhen Bay, was officially created in 1984 and designated as a national nature reserve in 1988. It belongs to the East Asian monsoon region with humid subtropical climate. The annual average temperature of 22.4 °C and average annual rainfall of 1700–1900 mm are conducive to the development of various microbial species (Meng & al. 2013). During our ongoing survey of anamorphic fungi associated with mangroves in China, we collected a new species representing *Beltrania* in the reserve.

Materials & methods

Isolation & morphological analysis

Specimens of dead leaves were collected from Futian Mangrove Nature Reserve, Shenzhen, Guangdong Province, China, and returned to the laboratory in plastic bags. The samples were incubated in plastic boxes lined with moistened tissue paper at room temperature for one week. Tissue pieces (5 × 5 mm) were randomly taken from the leaf and surface-sterilized by consecutively immersing in 75% ethanol solution for 1 min, 5% sodium hypochlorite solution for 30 s, and then rinsing three times in sterile distilled water for 1 min. The pieces were dried with sterilized paper towels and then placed on potato dextrose agar (PDA) (Cai & al. 2009). All the PDA plates were incubated at 25 °C for 2–4 d, and subcultures from the colony margins were inoculated

TABLE 1. Details of strains and sequences of *Beltrania* and related genera included in the phylogenetic analyses. New sequences are in bold; ex-(epi)type isolates are marked with ^T.

SPECIES	VOUCHER	GENBANK ACCESSION NUMBERS	
		ITS	LSU
<i>Beltrania dushanensis</i>	GZCC18-0020	MN252875	MN252882
<i>Beltrania krabiensis</i>	MFLUCC 16-0257 ^T	MH275048	MH260280
<i>Beltrania pseudorhombica</i>	CPC 23656 ^T	KJ869158	KJ869215
<i>Beltrania querna</i>	CBS 126097	MH864016	MH875474
	CBS 122.51	MH856775	MH868293
<i>Beltrania rhombica</i>	CPC 27482	KX519515	KX519521
	CBS 123.58	MH857718	MH869260
	strain 10353	—	AB496423
	CBS 121.50	—	MH868082
<i>Beltrania shenzhenica</i>	SAUCC 0061 ^T	MW784619	MW784621
	SAUCC 0065	MW784620	MW784622

SPECIES	VOUCHER	GENBANK ACCESSION NUMBERS	
		ITS	LSU
<i>Beltrania sinensis</i>	JS43	MN077366	MN077266
	JS42	MN077365	MN077265
	JS260	MN077364	MN077264
	JS101	MN077363	MN077263
<i>Beltraniella acaciae</i>	CPC 29498 ^T	KY173389	KY173483
<i>Beltraniella botryospora</i>	TUFC 10083 ^T	—	AB496426
<i>Beltraniella brevis</i>	GZCC 18-0081	MN252877	MN252884
	GZCC 18-0082	MN252876	MN252883
<i>Beltraniella carolinensis</i>	IFO 9502	—	DQ810233
<i>Beltraniella endiandrae</i>	CPC 22193 ^T	KJ869128	KJ869185
<i>Beltraniella fertilis</i>	MFLUCC 17-2137	MF580247	MF580254
	MFLUCC 17-2138	MF580248	MF580255
<i>Beltraniella humicola</i>	CBS 203.64	MH858416	MH870044
<i>Beltraniella pandanicola</i>	MFLUCC 18-0121 ^T	MH275049	MH260281
<i>Beltraniella portoricensis</i>	NFCCI 3993	KX519516	KX519522
	CBS 856.70	MH859981	MH871777
<i>Beltraniella pseudoportoricensis</i>	CBS 145547	MK876377	MK876416
<i>Beltraniella ramosiphora</i>	MFLUCC 17-2582 ^T	MG717500	MG717502
<i>Beltraniella thailandica</i>	MFLUCC 16-0377 ^T	MH275050	MH260282
<i>Beltraniopsis longiconidiophora</i>	MFLUCC 17-2139 ^T	MF580249	MF580256
	MFLUCC 17-2140	MF580250	MF580257
<i>Beltraniopsis neolitseae</i>	CPC 22168 ^T	KJ869126	KJ869183
<i>Beltraniopsis</i> sp.	TUFC 10081	—	AB496424
<i>Castanediella couratarii</i>	CBS 579.71	MH860269	MH872031
<i>Hemibeltrania cinnamomi</i>	NFCCI 3695	KT119564	KT119565
	NFCCI 3997	KX519517	KX519523
	MFLUCC 17-2141	MF580251	MF580258
<i>Porobeltraniella porosa</i>	NFCCI 3994	KX519518	KX519524
	NFCCI 3995	KX519519	KX519525
	NFCCI 3996	KX519520	KX519526
<i>Pseudobeltrania cedrelae</i>	COAD 2098 ^T	MG559548	MG559558
	PF 9	MG559552	MG559562
<i>Pseudobeltrania ocoteae</i>	CPC 26219 ^T	KT950856	KT950870
<i>Subramaniomyces fusisaprophyticus</i>	CBS 418.95	EU040241	EU040241
<i>Subsessila turbinata</i>	MFLUCC 15-0831 ^T	KX762288	KX762289

onto new PDA plates. Colonies at 7 d and 15 d were photographed using a PowerShot G7X mark II digital camera. Fungal micromorphological structures were observed and photographed using Olympus SZX10 stereomicroscope and Olympus BX53 microscope, both fitted with Olympus DP80 high definition colour digital cameras. All fungal strains were stored at 4 °C in 10% sterilized glycerin for further studies. The specimens are deposited in the Herbarium of Plant Pathology, Shandong Agricultural University, Taian, Shandong, China (HSAUP). Ex-type cultures are deposited in the Shandong Agricultural University Culture Collection, Taian, Shandong, China (SAUCC).

DNA extraction, PCR amplification, sequencing

Genomic DNA was extracted from colonies grown on PDA based on a modified CTAB method (Doyle & Doyle 1990). The nuclear ribosomal internal transcribed spacer (ITS) was amplified and sequenced using the primer pair ITS4/ITS5 (White & al. 1990), and the large subunit ribosomal RNA gene (LSU) using the primer pair LROR/LR5 (Vilgalys & Hester 1990, Glass & Donaldson 1995).

PCR was performed using an Eppendorf Mastercycler Thermocycler. The DNA was amplified in 25 µL reaction volumes containing 12.5 µL Green Taq Mix, 1 µL of each forward and reverse Biosune primer (10 µM), and 1 µL template genomic DNA in amplifier, and adjusted with distilled deionized water to a total 25 µL volume. PCR parameters followed were 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and a 1-min extension at 72 °C and ending with a final 10-min elongation at 72 °C. PCR products were estimated visually by staining with GelRed after 1% agarose gel electrophoresis. Sequencing was performed bi-directionally by BioSune Co. Ltd. (Shanghai). Consensus sequences were obtained using MEGA v. 7 (Kumar & al. 2016). All sequences generated in this study were deposited in GenBank (TABLE 1).

Sequence alignment & phylogenetic analysis

The quality of our amplified nucleotide sequences was checked and combined by MEGA v. 7 (Kumar & al. 2016), and reference sequences were retrieved from the National Center for Biotechnology Information (NCBI). Sequences were aligned using MAFFT v. 7.310 (Kato & al. 2019) and manually corrected using MEGA v. 7.

The combined gene regions were phylogenetically analyzed using Maximum-Likelihood (ML) and Bayesian Inference (BI). RaxML analyses (using RaxML v. 8.2.9) and Bayesian analyses (using MrBayes v. 3.2.6) were run on the CIPRES Science Gateway Portal (Miller & al. 2012). Evolutionary models were calculated using MrModeltest v. 2.3 (Nylander 2004) selecting the best-fit model for each data partition according to the Akaike criterion. For ML analyses the default parameters were used and bootstrap support (BS) was carried out using the rapid bootstrapping algorithm with the automatic halt option. Bayesian analyses included two parallel runs of 5,000,000 generations with the stop rule option and a sampling frequency set to each 1000 generations. The 50% majority rule consensus trees and posterior

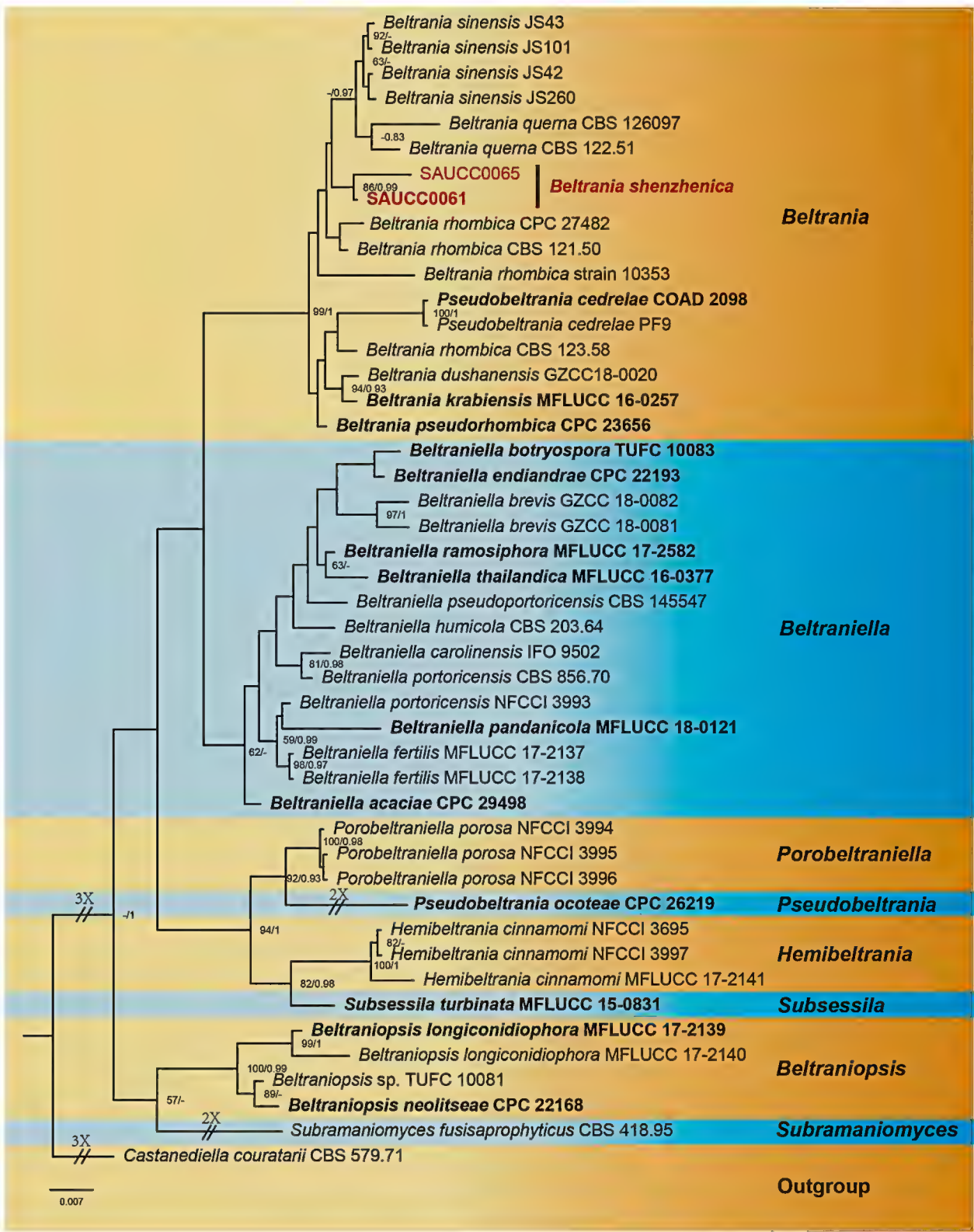


FIG. 1. Phylogram of *Beltrania* and related genera, generated from Bayesian analysis based on combined ITS and LSU sequence genes. Bootstrap support values are shown as ML >50% first, followed by BI >0.90. Some branches were shortened to fit the page – these are indicated by two diagonal lines and a correction factor indicating the full length. Ex-type/ex-epitype strains are in bold. Newly generated sequences are indicated in red.

probability (PP) values were calculated after discarding the first 25% of the samples as burn-in. The resulting trees were plotted using FigTree v. 1.4.2 (<http://tree.bio>).

ed.ac.uk/software/figtree) and edited with Adobe Illustrator CS v. 5. The individual gene datasets were assessed for incongruence before being concatenated by checking their individual phylogenies for conflicts between clades with significant ML and BI support (Mason-Gamer & Kellogg 1996, Wiens 1998).

Phylogenetic results

The dataset comprised 46 sequences representing 29 species including *Castanediella couratarii* (CBS 579.71) as the outgroup. The final alignment comprised a total of 1549 characters of the combined ITS and LSU including gaps, ITS: 1–940 and LSU: 941–1549. Of these characters, 1256 were constant, 152 parsimony-uninformative and 141 parsimony-informative. For the BI and ML analyses, the substitution model GTR+I+G for ITS and LSU were selected and incorporated into the analyses. The topology of Bayes tree confirmed the tree topologies obtained from the ML analyses, and therefore, only the Bayes tree is presented (FIG. 1). In this tree, our two sequences formed a distinct clade. Therefore, we determined that our strains belonged to a novel species of *Beltrania*.

Taxonomy

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FIG. 2

MB 839268

Differs from *Beltrania querna* by its wider conidia and from *B. rhombica* by its longer conidia.

TYPE: China, Guangdong Province, Shenzhen, Futian Mangrove Nature Reserve, on dead leaves of a broadleaf tree, 14 July 2020, Z.X Zhang (Holotype, HSAUP 0061; ex-type living culture SAUCC 0061; GenBank MW784619, MW784621).

ETYMOLOGY: in reference to the city where the type was found.

COLONIES on PDA at 25 °C in darkness, increasing in diameter by 11–15 mm/d, surface greyish white to black, flat, dense, reverse black to dark black. Mycelium partly superficial and partly immersed. Stroma absent. Setae erect, brown, thick-walled, septate, straight to flexuous, conical at the apex, slightly swollen at basal cell. Conidiophores 58–88 × 3–5 µm, aggregated in dense fascicles, pale brown, cylindrical, septate, unbranched, straight to variously curved, proliferating sympodially at apex. Conidiogenous cells terminal, integrated, subhyaline, smooth, holoblastic, polyblastic, with several flat tipped denticles. Separating cells 8–16 × 5–7 µm, subhyaline, finely roughened, with several apical, flat-tipped denticles. Conidia 28–33 × 8.5–12 µm (including apical appendage), biconic, aseptate, solitary, acropleurogenous, subhyaline to pale brown, with distinct granules,



FIG. 2. *Beltrania shenzhenica* (holotype, HSAUP 0061). a, b. Surface and reverse of colony on PDA; c. Mycelium on PDA; d. Setae; e, f. Conidiophores, separating cells, conidiogenous cells, and conidia; g–i. Separating cells; j–l. Conidia. Scale bars: d–l = 10 μ m.

without median transverse band, apical appendage 5–9 μ m long, tapering to an acutely rounded tip, smooth, without a mucilaginous sheath.

ADDITIONAL SPECIMEN EXAMINED: CHINA, GUANGDONG PROVINCE, Shenzhen, Futian Mangrove Nature Reserve, on dead leaves of a broadleaf tree, 14 July 2020, Z.X. Zhang (HSAUP 0065; living culture SAUCC 0065; GenBank MW784620, MW784622).

COMMENTS – Based on phylogenetic analysis, our *Beltrania shenzhenica* sequences grouped together with *B. querna* and *B. rhombica*, but they formed a distinct clade. Morphologically, *B. shenzhenica* is most similar to *B. querna*

and *B. rhombica* in conidial shape, but *B. querna* differs by its narrower conidia (6–8 µm wide; Harkness 1884), and *B. rhombica* differs by its shorter conidia (25–26 µm long; Penzig 1882).

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